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EFFECT OF DIETARY LINOLEIC ACID CONTENT OF PLATELET AGGREGATION AND CALCIUM SENSITIVITY AND ON MICROVISCOSITY OF PLATELET MEMBRANES IN RATS

V. G. Pinelis, V. S. Poleshchuk, A. I. Kosikov, and Kh. M. Markov

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Diets rich in saturated or polysaturated fatty acids (PUFA) have opposite actions on platelet (PL) function in man [5, 8-10] and experimental animals [12, 13, 15]. It is claimed that the action of such diets is based on food modification of the PL spectrum of PUFA, which are precursors of the eicosanoids in phospholipid membranes, and this leads to changes in the synthesis of compounds (such as thromboxane A_2, prostaglandins E_1 and E_2, etc.) which have a powerful effect on the development of PL aggregation [12]. Meanwhile reception of the inducer of PL aggregation and their response to it depend on the state of the membrane, in which lipids are one of the principal structural components. However, the concrete mechanisms of the effect of food fatty acids on PL have not been finally established. There are contradictory data, due to differences in the duration of exposure, the species of the experimental animals [15], the quantity and quality of PUFA in the diet, and so on.

The aim of this investigation was to study the characteristics of aggregation of PL and their sensitivity to Ca^{++} and the microviscosity of PL membranes in rats receiving diets differing in their linoleic acid (LA) content.

EXPERIMENTAL METHOD

Three semisynthetic diets, equal in calorific value but differing in their LA (18:2n6) content: diet I) 0.1 calorie % (cal. %) of LA; II) 9.0 cal. % of LA; diet III) 16 cal. % of LA. The diets were made up by V. V. Atrokhov, on the staff of the authors' laboratory, on a basis of data in the literature [1] and they included a fat-free basis together with a mixture of water-soluble vitamins in casein with the addition of fatty components: for diet I) 11% of hydrogenated sunflower oil (Salomas) and 1% of a mixture of fat-soluble vitamins in Salomas; for diet II) 4% of sunflower oil, 5% of lard, 2% of Salomas, and 1% of a mixture of fat-soluble vitamins in sunflower oil; for diet III) 10% of sunflower oil, 1% of lard, and 1% of a mixture of fat-soluble vitamins in sunflower oil. The mineral composition of the diet was supplied by the addition of Jones—Foster salt mixture [1] supplemented by fluorine (0.86 mg/100 g of dry food mixture). To prepare the diets all the ingredients were finely ground, and mixed uniformly to obtain a homogeneous powdery mass, which was kept at −20°C. If necessary

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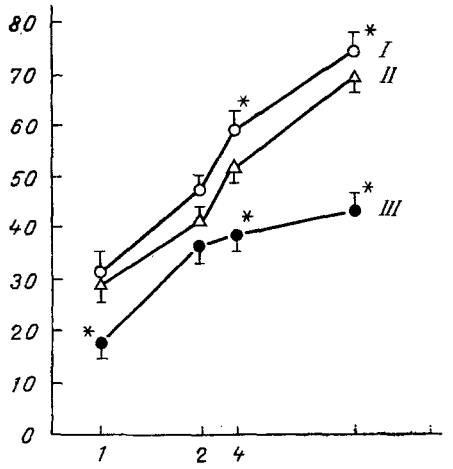

Fig. 1. ADP-induced aggregation of washed PL, obtained from blood of rats kept on diets differing in their LA content (ADP concentration 1-10 μM). Abscissa, logarithm of dose of ADP (in μM); ordinate, transmittance of sample (in %). I, II, III) Diets.

boiling water was added to the dry mixture, which was then made into a dough, from which cakes were baked for the animals. Male Wister-Kyoto rats, whose mothers had been kept for the last week of pregnancy and for 1 month of the period of lactation on the above-mentioned diets, were divided into three groups in accordance with the diets received, and they were kept on the above diets until the age of 18 weeks. The animals were given food and water ad lib.

Washed PL were isolated by a modified method [11]. The animals were deprived of food on the night before the experiment. Next day, under superficial ether anesthesia, the abdominal aorta was punctured and blood collected in polyallomer tubes containing citrate buffer (6:1 by volume; 93 mM sodium citrate, 7 mM citric acid, 0.14 M glucose, pH 6.5). PL-enriched plasma was obtained by centrifugation at 175 g for 13 min at room temperature. Subsequent operations were carried out at 4°C. The plasma thus obtained was treated with an equal volume of citrate buffer, centrifuged for 7 min at 1400g, and the PL residue was washed twice in the following buffer: 136.9 mM NaCl, 2.68 mM KCl, 1.05 mM $MgCl_2$, 11.9 mM $NaHCO_3$, 0.42 mM NaH_2PO_4, 5.5 mM glucose, 5 mM HEPES, 0.1 mg/ml of apyrase, and 0.35% solution of bovine serum albumin (BSA) (pH 6.5). The PL residue was finally resuspended in incubation buffer (IB), similar in composition to the washing buffer, but without apyrase and BSA (pH 7.35). The number of washed PL was determined with a C-209 platelet counter (Nedata, Sweden). PL were aggregated on a two-channel aggregometer (Payton, Canada). For this purpose 0.5 ml of a PL suspension, containing 10^8 cells, was poured into the aggregation cuvette, kept for 2-3 min at 37°C with constant stirring (1000 rpm), and 2 mM Ca^{++} was added 1 min before addition of the aggregation inducer. ADP (1-10 μM) and the Ca^{++}-ionophore A_{23187} (2 μM) were used as aggregation inducers. The maximal amplitude of aggregation (in %) and the velocity constant of aggregation were determined from the induced aggregation curves. The last parameter was calculated by the equation:

$$K = \frac{\operatorname{tg}\alpha}{V} \cdot \frac{A}{H},$$

where α is the angle of slope of the aggregation curve, V the recording speed, A the maximal amplitude of aggregation, and H the transmittance of the 0-th sample. The sensitivity of PL in Ca^{++} was estimated as the value of pCA_{50}, the negative logarithm of the Ca^{++} concentration (in mM) at which A_{23187} induced aggregation of half the maximal degree. Ca^{++} was added in a final concentration of 0.2-2.0 mM. The value of pCa_{50} was calculated by the method of least squares, by approximating the dose–effect curve by the straight line $y = ax + b$ (a and b are coefficients, x denotes the Ca^{++} concentration).

To measure the microviscosity of the PL membrane lipids the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPTH) was used in a final concentration of 10^{-6} M. Before the experiment the solution of DPHT in tetrahydrofurane ($2 \cdot 10^{-3}$ M) was diluted in IB to a concentration of $2 \cdot 10^{-6}$ M. Next 1 ml of the PL suspension ($6 \cdot 10^8$ cells) was mixed with a solution of DPHT and incubated for 1 h at 20°C (medium without DPHT was added to the control samples). After incubation, the PL were washed to remove the probe by centrifugation (1100g, 15 min) and the residue was resuspended in twice the volume of IB up to $3 \cdot 10^8$ cells. Fluorescence of DPHT was measured on a Hitachi 650-60 spectrofluorometer (Japan), with an excitation wavelength of 360 nm and with slits 4 nm wide. The microviscosity of the membrane lipids was calculated from data on the change in the degree of polarization of fluorescence of DPHT (P)

TABLE 1. Aggregated Power of PL, Sensitivity to Ca^{++}, and Microviscosity of Platelet Membranes in Rats on Diets Differing in Their LA Content

Diet	Number of animals	PL aggregation, % of transmittance		pCa_{50}	Microviscosity, II
		ADP (10 μm)	A_{23187} (4 μm)		
I	11	73,3±1,2** (0,7±0,05)	71,0±1,6** (2,3±0,6)	0,6±0,03**	3,2±0,02**
II	16	70,0±1,5 (0,7±0,07)	65,0±1,2 (3,3±0,3)	0,5±0,02	3,0±0,02
III	8	45,5±1,6* (0,2±0,02)*	65,0±1,8 (1,4±0,4)*	0,4±0,02*	2,8±0,03

Legend. Velocity constant of aggregation (in min^{-1}) shown between parentheses.
*p < 0.05 compared with diets I and II;
**p < 0.05 compared with diets II and III.

by the equation [14]:

$$\eta = \frac{2P}{0,46 - P}(\pi), \text{ where } P = \frac{\Delta F_{\parallel} - \Delta F_{\perp}}{\Delta F_{\parallel} + \Delta F_{\perp}}.$$

The results were subjected to statistical analysis in the usual way. The significance of differences was determined by Student's t test.

EXPERIMENTAL RESULTS

Data on the effect of diets containing different amounts of LA on the aggregating power of PL are given in Fig. 1 and Table 1. With an increase in the LA content of the diet (on transition from diet I to diet III) the maximal amplitude of ADP-induced aggregation gradually decreased. As regards changes in PL aggregation after addition of A_{23187}, the amplitude of aggregation was significantly higher on diet I than on diets II and III. However, there were no differences between the last two diets. The velocity constant of aggregation induced by ADP or A_{23187} was least on diet III, and diets I and II caused no marked changes in this parameter. Reduction of the various parameters of PL aggregation under the influence of diets containing an excess of LA has been found in many investigations both in man [8-10] and in experimental animals [13, 15]. Meanwhile the present investigation showed that absence of LA in the diet (diet I) leads to increased PL aggregation. At the same time, unlike Hornstra, et al. [9], we found no strict relationship between the degree of changes in PL aggregation and the LA intake with the food (diets II and III).

Considering the important role of Ca^{++} in the mechanisms of PL aggregation [2, 6], we investigated the sensitivity of PL to this cation during aggregation induced by A_{23187}. As Table 1 shows, pCa_{50} was increased on diet I compared with its value on diet III, but no significant differences were found in the changes in this parameter on diets I and II. Increased sensitivity to Ca^{++} on diet I, for which a lower concentration of this cation in the medium was required for the development of complete PL aggregation by comparison with diet III, may be connected with the reduced ability of the Ca^{++}-transport systems of PL to compensate for the influx of Ca^{++} into the cell after injection of the aggregation induced (as a result of which the concentration of free cytoplasmic Ca^{++} should increase), leading to intensification of PL aggregation.

Changes in the fatty acid composition of the phospholipids, which take place, according to data obtained in the authors' laboratory (in press), when diets differing in their LA content are used, may in turn have some effect on the structural state of the membrane, and may thereby determine changes in the functional state of PL [3, 7]. The use of the fluorescent probe DPHT, charcterizing the microviscosity of the lipid bilayer of PL membranes [14] [sic], revealed definite structural changes in the PL membranes of the rats on diets differing in their LA content. As Table 1 shows, diet I caused an increase in microviscosity of the lipids of the PL membranes, but in animals on diet III, on the contrary, this parameter was reduced. Changes discovered in the microviscosity of the lipids of the PC membranes may be due to the action of two factors: on the one hand, a disturbed ratio of saturated to unsaturated fatty acids, and on the other hand, the effect of cholesterol, which is a natural modulator of

microviscosity of PL membrane lipids [3, 4]. There is evidence to show that this ratio and the cholesterol concentration was increased [3] in animals on diets with a low LA content, whereas the opposite changes are found on diets rich in LA [3].

Diets with diametrically opposite LA content may thus possess antithrombogenic or thrombogenic properties on account of changes in the physicochemical characteristics of the PL membranes and sensitivity of PL to Ca^{++}.

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TERMINATION OF ANOXIA BY APNEIC OXYGENATION WITH EXTRAPULMONARY MEMBRANE CO_2 REMOVAL

V. I. Skorik, T. M. Malikova,
L. F. Boltovskaya, B. M. Zelikson,
E. S. Safonova, and E. V. Perezhogin

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The method of apneic oxygenation with extrapulmonary CO_2 elimination is used in the treatment of respiratory failure [3-10]. The method essentially enables the body to carry out gas exchange while the lungs remain relatively at rest, with favorable effects on their treatment. The combined use of pulmonary and extrapulmonary routes of CO_2 elimination and O_2 intake also has advantages. Oxygen insufflation into the lungs under low pressure, combined with oxygenation of the blood in a membrane oxygenator (MO), ensures an adequate oxygen supply to the body, and excludes barotrauma and other detrimental consequences of artificial ventilation of the lungs (AVL). Extrapulmonary removal of CO_2 with the aid of a MO can be done with small perfusion volumes through this device, so that the safe periods of perfusion can be considerably lengthened. Some aspects of the gas exchange when anoxia is terminated by this method have been inadequately explained.

This paper describes a study of the gas exchange of animals during apneic oxygenation and extrapulmonary elimination of CO_2, using an improved Sever MO [2] with small perfusion volumes.

EXPERIMENTAL METHOD

Three series of experiments were carried out on 34 dogs of both sexes, weighing 14-18 kg. After premedication with pentobarbital (20 mg/kg) anesthesia was maintained by periodic intravenous injections of hexobarbital (10 mg/kg) and heparin was given in an initial dose

P. A. Kupriyanov Postgraduate Surgical Clinic, S. M. Korov Military Medical Academy, Leningrad.

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